BIODIVERSITAS
Volume 25, Number 8, August 2024
Pages: 3422-3430

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250813

The antimicrobial and antioxidant potentials of *Annona* species (*A. muricata*, *A. squamosa*, and *A. reticulata*) through leaf infusions

NURMIATI NURMIATI[1,♥], PERIADNADI PERIADNADI[1], SONIA FARMILA SYAHRIL[1], TRI WIDYA EDELWIS[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas. Jl. Raya Unand, Limau Manis, Padang 25163, West Sumatra, Indonesia. Tel./fax.: +62-751-71671, ♥email: nurmiati@sci.unand.ac.id, periadnadiperiadnadi@gmail.com
[2]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Raja Ali Haji Maritime. Jl. Politeknik Senggarang Km. 24, Tanjung Pinang 29115, Riau, Indonesia

Manuscript received: 30 March 2024. Revision accepted: 9 August 2024.

Abstract. *Nurmiati N, Periadnadi P, Syahril SF, Edelwis TW. 2024. The antimicrobial and antioxidant potentials of* Annona *species (*A. muricata, A. squamosa, *and* A. reticulata*) through leaf infusions. Biodiversitas 25: 3422-3430.* This study aimed to determine the antimicrobial and antioxidant potential of infusions derived from dried leaves of Soursop (*Annona muricata* L.), Sugar Apple (*Annona squamosa* L.), and Custard Apple (*Annona reticulata* L.). Using a nested pattern experimental method, this research aimed to evaluate the potential of these plant extracts in inhibiting microbial growth and antioxidant properties. The experiment used in this research is a nested experimental design with 2 factors and 3 replications, where factor A represents the extract type, while factor B represents the test microorganism. The antibacterial assay was conducted using the disk diffusion and dilution methods against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) are determined. Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, while Total Phenolic Content (TPC) and carotenoid levels were measured using Folin-Ciocalteu and spectrophotometric methods, respectively. The results showed that the infusion of dried custard apple leaves had the highest growth inhibition against *S. aureus* ATCC 29213, with a zone of inhibition of 10.65 mm. However, these effects were not significantly different for *E. coli* American Type Culture Collection (ATCC) 25922 and *C. albicans*. The dried custard apple leaf infusion showed an inhibition rate of 48.58% against *S. aureus*, compared to the positive control chloramphenicol (0.1 mg/mL), and inhibition rates of 24.57% against *E. coli* and 28.70% against *C. albicans*, compared to the positive control fluconazole (0.1 mg/mL). The results revealed that the Minimum Inhibitory Concentration (MIC) of soursop and custard apple leaf extracts against *S.* was 6.25%, with a Minimum Lethal Concentration (MLC) of 12.5%. The MIC of sugar apple leaf extract against *S. aureus* was 6.25%, with an MLC of 25%. The antioxidant activity of the soursop leaf extract had the highest antioxidant activity, with the IC_{50} value of 38.56 µg/mL. The custard apple leaf extract had the highest polyphenol content (38.97 mgGAE/mL). The fresh sugar apple leaf extract emerged as the leader in carotenoid content, recording an impressive 548.84 µmol/g. This research revealed the potential bioactivity of the infusions derived from dried leaves of Soursop, Sugar Apple, and Custard Apple for antimicrobial efficacy and potent antioxidant properties. Therefore, they might have the potential as novel therapeutic agents and nutraceuticals.

Keywords: *Annona*, antimicrobial, antioxidant, infusion, leaves

Abbreviations: MIC: Minimum Inhibitory Concentration; MLC: Minimum Lethal Concentration; SDA: Sabouraud Dextrose Agar; MHA: Mueller Hinton Agar; NA: Nutrient Agar; PDA: Potato Dextrose Agar; MHB: Mueller Hinton Broth; SDB: Sabouraud Dextrose Broth; ATCC: American Type Culture Collection; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TPC: Total Phenolic Content

INTRODUCTION

Antimicrobial resistance is a global public health problem that needs to be solved. Antimicrobial resistance causes several problems, such as increasing morbidity and mortality, costs and length of treatment, and side effects from the use of multiple drugs in high doses. Alternatives that can be used are antimicrobials derived from plant materials. There are four main groups of antimicrobial compounds produced by plants: phenolics and polyphenols, terpenoids and essential oils, lectins and polypeptides, and alkaloids (Hashem et al. 2023). This phenolic compound is a bioactive compound with antimicrobial and antioxidant properties. One of the plant families that contain phenolic compounds is the Annonaceae family, such as Soursop (*Annona muricata* L.), Sugar Apple (*Annona squamosa* L.), and Custard Apple (*Annona reticulata* L.).

The search for novel therapeutic agents and nutraceuticals has led to a surge in research exploring the bioactive properties of natural sources. Among these, plants have emerged as promising reservoirs of bioactive compounds with potential health benefits. In this context, the genus *Annona*, comprising species like Soursop (*Annona muricata* L.), Sugar Apple (*Annona squamosa* L.), and Custard Apple (*Annona reticulata* L.), has garnered considerable attention due to its rich phytochemical profile and traditional medicinal uses (Kavithaa et al. 2016; Haruna et al. 2022; Vishvakarma et al. 2023).

Soursop (*Annona muricata* L.) has been utilized in herbal medicine. Its leaves contain tannins, phytosterols, ca-oxalate, murisine alkaloids, and sesquiterpene derivatives

(Ahmad et al. 2024). The soursop leaf is a remedy for ulcers, boils, seizures, acne, head lice, and diarrhea (Uchegbu et al. 2017). The soursop leaf juice inhibited the growth of *Escherichia coli* (Jemikalajah et al. 2021).

Sugar apple leaves (*Annona squamosa* L.) have been traditionally utilized as a remedy for coughs, rheumatism, digestive tract disorders (diarrhea, dysentery, flatulence), skin diseases (ulcers, boils, scabies), as well as for enhancing stamina and reducing fever. These leaves contain various bioactive compounds, including tannins, phenolics, polyphenols, glycosides, saponins, phytosterols, alkaloids, and flavonoids. These compounds are natural antimicrobials and antioxidants (Kumar et al. 2021). The methanol extract and ethyl acetate extract of sugar apple leaves inhibit the growth of *E. coli* and *Staphylococcus aureus* (Ahmad Shiekh et al. 2021).

The custard apple (*Annona reticulata* L.) is traditionally used to treat epilepsy, dysentery, heart-related issues, constipation, bleeding, antibacterial infections, dysuria, fever, and stomach problems. The custard apple leaves contain alkaloids, amino acids, carbohydrates, flavonoids, glycosides, proteins, steroids, tannins, and phenolic compounds (Ngbolua et al. 2018). The methanol extract from custard apple leaves (*A. reticulata*) inhibits the growth of bacteria, such as *Staphylococcus aureus, Staphylococcus epidermidis, Proteus vulgaris, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Salmonella typhi*, with the highest inhibitory potential against *Bacillus subtilis* and *Escherichia coli* (Kumari et al. 2021).

Empirically, various methods exist for processing medicinal plants, including boiling fresh or dried leaves or simply infusion in hot water (Fonmboh et al. 2020). An alternative to processing medicinal plants is an infusion from dried leaves or herbal tea. Utilizing boiling water in the infusion process does not eliminate the phenolic compounds in dried leaves (Rohmah and Margareta 2023). In addition to being convenient, the drying process can extend the shelf life of herbal medicines.

Several species from Annonaceae are recognized for their antimicrobial and antioxidant (Chowdhury et al. 2021; dos Santos et al. 2023). However, comparative research has not been conducted on the antimicrobial and antioxidant activity of infusions from dried leaves, soursop, sugar apple, and custard apple leaves. Based on this gap in the literature, the author is interested in testing the antimicrobial and antioxidant activity of infusions of dried leaves of soursop (*A. muricata*), sugar apple (*A. squamosa*), and custard apple (*A. reticulata*) leaves.

This study evaluates the antimicrobial and antioxidant activities of infusions of dried soursop leaves, sugar apples, and custard apples. The findings from this study can serve as a foundation for further research on the bioactivities and properties of these plants. The antimicrobial activity and antioxidant potentials provide valuable insights into the therapeutic applications of *Annona* species, suggesting their potential use in pharmaceuticals or natural remedies for combating microbial infections and oxidative stress-related conditions. The isolation of the specific bioactive compounds responsible for their bioactivities is also needed. Moreover, a similar methodology and experimental approach could be applied to other parts of *Annona* plants or species within the genus.

MATERIALS AND METHODS

Study area

The research was conducted from April to June 2023 at the Microbiology Research Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia.

Laboratory equipment and materials

The laboratory equipment used included Petri dishes, test tubes, Erlenmayer, spirit lamp, test tube rack, measuring cup, dropper pipette, autoclave, incubator, hot plate, vortex, stirrer, digital scale, colony counter, tweezers, micropipette, spectrophotometer, calipers, disc paper. The materials used in this research include dry leaves of *A. muricata*, dry leaves of *A. squamosa*, dry leaves of *A. reticulata*, pure cultures of *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *C. albicans*, nutrient agar (NA) (Merck), Potato Dextrose Agar (PDA) (Merck), Mueller Hinton Agar (MHA) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), Mueller Hinton Broth (MHB) (Merck), Sabouraud Dextrose Broth (SDB) medium (Merck), alcohol 70% Onemed (Sigma), Follin-Ciocalteau reagent (Sigma), DPPH solution, Na_2CO_3 (Sigma), methanol p.a. (Merck), Vit C, Gallic acid (Sigma), Chloramphenicol (Sigma), and Fluconazole (Sigma).

Research methods

The method used in this research is a nested experimental design with 2 factors and 3 replication, where factor A represents the extract type, while factor B represents the test microorganism.

Procedures

Medium preparation

In separate Erlenmeyer flasks, 5 g of NA, 10 g of PDA, 9.5 g of MHA, 16.5 g of SDA, 5.5 g of MHB, and 7.5 g of SDB were dispensed and diluted with distilled water to a total volume of 250 mL. Subsequently, the solutions were heated on a hot plate until reaching the boiling point and then subjected to sterilization using an autoclave set at a temperature of 121°C and a pressure of 1 atm for 15 minutes (Nurmiati et al. 2018).

Preparation of test microbial suspension

Each pathogenic microbe was inoculated in a NaCl 0.9% solution using a needle, then homogenized with vortex until the turbidity was equivalent to McFarland's 0.5 standard solutions (Ahmed et al. 2021).

Antimicrobial activity test with diffusion and dilution methods

MHA (Mueller-Hinton Agar) and SDA (Sabouraud Dextrose Agar) were dispensed into separate Petri dishes containing approximately 15 mL of medium until they solidified. A sterile cotton swab spread a microbial suspension

evenly across the Petri dish surface. Subsequently, Each paper disc was aseptically placed into each dish using sterile tweezers and incubated for 24 hours. Additionally, 2 µL of extract was transferred to each disc. After incubation, observations and measurements of the diameter around each disc were carried out using a caliper to assess microbial inhibition zones. The positive controls, Chloramphenicol (0.1 mg/mL) and Fluconazole (0.1 mg/mL) were used for comparison.

Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) were determined using the Dilution Method. The extract was diluted in SDB/MHB medium to obtain concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.6%, 0.8%, 0.4%, 0.2%, and 0.1%. Subsequently, 1 mL of each test microbial suspension was added to separate test tubes. The tubes were then incubated at 37℃ for 24 hours. The turbidity of the solution was observed after 24 hours.

After incubation, 1 mL of the solution from a clear tube was transferred to the SDA/MHA medium. Subsequently, this plate was incubated for 24 hours. The determination of Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) was based on the lowest concentration in the dilution series that showed no growth of the test microbes after being cultured on the SDA/MHA medium (Balouiri et al. 2016).

Determination of antioxidant activity (IC50) using the DPPH method

The assessment of antioxidant activity was conducted using the DPPH free radical scavenging method, employing 1.1-Diphenyl-2-Picryl-Hydrazine (DPPH). The DPPH method was carried out following (Molyneux 2004). The infusion extract from dried leaves of soursop, sugar apple, and custard apple was reacted with a DPPH solution. The analysis of antioxidant activity was conducted using a Spectrophotometer at a wavelength of 517 nm (Kuppusamy et al. 2020).

Gallic acid standard curve

Standard solutions were prepared using various concentrations of gallic acid (0, 50, 100, 150, and 200 ppm). A mixture was prepared by mixing 1 mL of the standard gallic acid solution with 1 mL of Folin-Ciocaltaeu reagent and incubated for 5 minutes. 1 mL of Na_2CO_3 was added to the mixture. The volume was then adjusted to 10 mL using distilled water, followed by homogenization. The resulting solution underwent incubation for approximately ninety minutes. The absorbance value was subsequently measured at a wavelength of 725 nm (Herrera-Calderon et al. 2016).

Determination of Total Phenolic Content (TPC) by the Folin-Ciocalteu method

The quantification of phenolic compounds in the infusion extract of dried leaves from soursop, sugar apple, and custard apple was conducted by the Folin-Ciocalteu Assay. In the analysis of the samples, The infusion extract was mixed with Folin-Ciocalteu reagent at an equal volume at the 5-minute intervals. 1 mL of a 13% Sodium Carbonate solution was added to achieve a pH of 10 until the final volume reached 10 mL. The resulting mixture was then kept in darkness for 90 minutes before measuring its absorbance using a spectrophotometer set to a wavelength of 765 nm (Kahraman et al. 2019).

Determination of carotenoid

0.1 g of fresh soursop, sugar apple, and custard apple leaves were ground using a mortar. The resulting ground material is then extracted with 10 mL of 80% acetone and stirred until the carotenoids are dissolved. The extract is subsequently filtered using filter paper. The obtained filtrate is placed in a cuvette to measure carotenoid content with a spectrophotometer at 480 nm, 645 nm, and 663 nm wavelengths. After obtaining the absorbance values, the calculation of carotenoid content follows the formula proposed by (Viñas-Ospino et al. 2023).

$$\text{Carotenoid } (\mu\text{mol/L}) = \frac{(A480+(0{,}114 \times A663))-(0{,}638 \times A645)) \, v \, .10^3}{112{,}5 \times W}$$

Where:
A480 : Absorbance at wavelength 480 nm
A645 : Absorbance at wavelength 645 nm
A663 : Absorbance at wavelength 663 nm
V : Extract volume (mL)
W : Sample weight (g)

Data analysis

The data from the diffusion method underwent analysis using the nested pattern approach. In cases where significant differences were observed among the treatments, subsequent data analysis was conducted using the DMRT test at a significance level of 5%. Conversely, the descriptive study was employed to assess antioxidant activity, total phenolic content, and total carotenoid content based on results obtained from the dilution method (Wang et al. 2023).

RESULTS AND DISCUSSION

Based on the results of the antimicrobial test of infusion of dried soursop, sugar-apple, and custard apple leaves using the disc diffusion method were presented in Table 1. This table showed that three extracts had significantly different effects on *S. aureus*, with the largest inhibition zone observed in the infusion extract of dried soursop leaves (10.65 mm). However, there was no significant difference in the diameter of inhibition on *E. coli* and *C. albicans*, as no inhibition zones were observed. It indicated that the infusion extracts from dried soursop (*A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*) leaves can inhibit the growth of *S. aureus* but do not show inhibitory effects on the growth of *E. coli* and *C. albicans.*

Table 1. Diameter of inhibition of three *Annona* species against test microorganisms

Extract	Antimicrobial zone diameter (mm)		
	E. coli	***S. aureus***	***C. albicans***
Positive control	22.33	24.42	20.9
Soursop leaves (Dried infusion)	6.03[a]	8.74[b]	6.00[a]
Sugar-apple leaves (Dried infusion)	6.00[a]	7.10[c]	6.00[a]
Custard apple leaves (Dried infusion)	6.00[a]	10.65[a]	6.00[a]

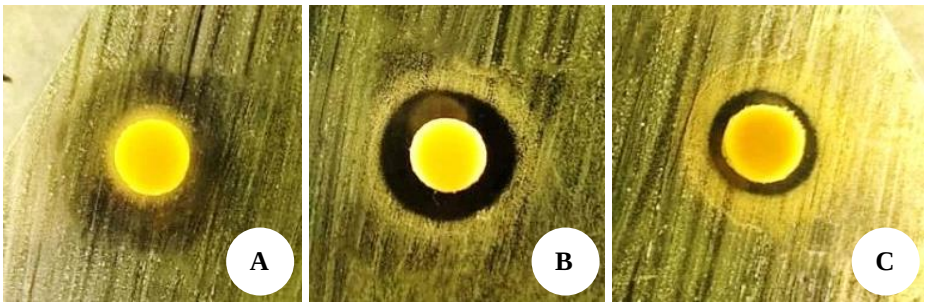


Figure 1. Antimicrobial activity of dried infusion extracts from Soursop leaves *(A. muricata*), Sugar-Apple (*A. squamosa*), and Custard Apple (*A. reticulata*) against *S. aureus*. A. Custard Apple leaves; B. Soursop leaves; C. Sugar-Apple leaves

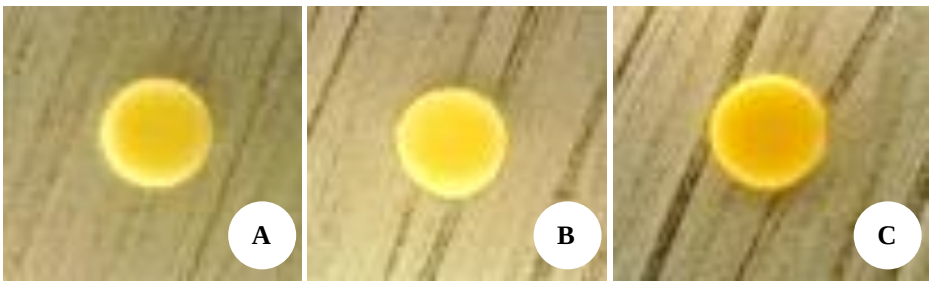


Figure 2. Antimicrobial activity of dried infusion extracts from soursop *leaves (A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*) against *E. coli*. A. Custard Apple leaves; B Soursop leaves; C. Sugar-Apple leaves

The infusion extract from custard apple leaves demonstrated the largest inhibition zone (10.65 mm), followed by the infusion extract from soursop leaves (8.74 mm) and sugar-apple leaves (7.10 mm). The varying diameters of the inhibition zones among the extract are attributed to active compounds within each extract and the strain of test microorganisms. Antibacterial activity is influenced by multiple factors, including extract concentration, the composition of antibacterial compounds, the diffusion capacity of the extract, and the type of bacteria (Ramyabharathi et al. 2020).

The antibacterial mechanisms of each secondary metabolite compound vary. Alkaloid compounds disrupt the bacterial cell peptidoglycan components, preventing the formation of the cell wall layer and leading to cell death. Flavonoid compounds, as antibacterials, form complex compounds with soluble extracellular proteins. This interaction can damage the bacterial cell membrane, followed by the release of intracellular compounds (Weng et al. 2024).

The three extracts inhibit the growth of *S. aureus* because *S. aureus*, as a Gram-positive bacterium, possesses a polar cell wall, making it easily penetrated by polar compounds such as alkaloids, flavonoids, and tannins. In Gram-positive bacteria, teichoic acid is connected to peptidoglycan through covalent bonds. Teichoic acid is hydrophilic (water-soluble) and is a medium for transporting positively charged ions in and out of the cell wall (Shrestha et al. 2023). The water-soluble nature of teichoic acid makes Gram-positive bacteria's cell walls more polar than Gram-negative bacteria's.

The inhibition zone of infusion of soursop leaves against *E. coli* was 6.03 mm, but sugar apple and custard apple leaf infusions did not inhibit the growth of *E. coli.* Figure 2 shows that the absence of an inhibition zone in sugar apple and custard apple leaf infusions may be due to the active compounds in the extract and the extraction process. The hot water infusion breaks ester and glycoside bonds in phytochemical compounds, extracting them into the water. Since polar solvents dissolve polar compounds, water, being a polar solvent, will dissolve polar

phytochemicals like alkaloids, flavonoids, saponins, and tannins (Bitwell et al. 2023).

The absence of an inhibition zone in the *E. coli* treatment is also influenced by the structure of the bacterial cell wall. The cell wall of *E. coli* has a non-polar lipid layer, making polar compounds challenging to penetrate. *E. coli* is a Gram-negative bacterium with three layers of cell walls, i.e., lipopolysaccharide, protein, and phospholipid (Mariychuk et al. 2020). The bacterial cell wall's structural composition significantly impacts antibacterial compounds' penetration, binding, and effectiveness (Rajendran and Mani 2020).

The leaf infusion of soursop, sugar apple, and custard leaves did not inhibit the growth of *C. albicans*. It might be due to the complex structure of the cell wall of *C. albicans*. The cell wall of *C. albicans* comprises polysaccharides (mannan, glucan, chitin), proteins, and lipids, with a membrane containing sterols beneath it (El-Belely et al. 2021). The absence of an inhibition zone is also influenced by the extraction method and the concentration of the extract. The extraction method used in this research is the infusion method. As a solvent, water may result in incomplete extraction of active compounds (Rohmah and Margareta 2023). The low inhibitory capability may be attributed to the small amount of dissolved active compounds.

The diameter of the inhibition zone of chloramphenicol against *S. aureus* is 22.33 mm. Compared to the positive control of chloramphenicol, the growth inhibition of infusion of dried custard apple (*A. reticulata*) is 48.58%, the dried soursop (*A. muricata*) is 43.48%, and the dried custard apple (*A. squamosa*) is 32.91%. The diameter of the inhibition zone for the positive control chloramphenicol against *E. coli* is 24.42 mm. Compared to the controls, the growth inhibition of infusion of dried custard apple *(A. reticulata*) is 24.57%, the dried soursop *(A. muricata)* is 24.73%, and the dried custard apple *(A. squamosa*) is 24.57%. The mechanism of action of the chloramphenicol antibiotic is inhibiting the peptidyl transferase enzyme, which plays a role in the formation of peptide bonds in the bacterial protein synthesis process (Tiwari et al. 2018).

The diameter of the inhibition zone for the positive control fluconazole against *C. albicans* is 20.29 mm. Compared to the positive control fluconazole, the growth inhibition of the infusion of dried custard apple (*A. reticulata*) is 28.70%, the dried soursop (*A. muricata*) is 28.70%, and the dried sugar apple (*A. squamosa*) infusion is 28.70%. Fluconazole is an antifungal of the triazole group that acts as a potent inhibitor of ergosterol biosynthesis by inhibiting 14-α-demethylase, a microsomal cytochrome P450 enzyme in the fungal membrane (Medda et al. 2015).

Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC)

Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) values of leaf infusion extract of soursop, custard apple, and custard apple were conducted using the dilution method. Based on Table 3, the results show that the MIC value of the leaf infusion extract of soursop was 6.25% and a Minimum Lethal Concentration (MLC) value of 12.5%. The MIC and MLC values of the sugar apple leaf infusion were 6.25% and a Minimum Kill Rate value of 25%. The MIC and the MLC values of the leaf infusion extract of Custard apple were 6.25% and 12.5%, respectively.

The presence of inhibitory and bactericidal effects is related to bioactive compounds in the extracts, such as alkaloids, flavonoids, tannins, terpenoids, and phenols. Based on antibacterial activity, antibacterial substances are classified into two types, i.e., bacteriostatic activity (inhibiting bacterial growth) and bactericidal activity (killing bacteria). The activity of an antibacterial substance in inhibiting or killing the growth of microorganisms depends on the concentration and type of antibacterial agent. The higher the concentration, the higher the antibacterial activity of the drug against bacteria (Lingaraju et al. 2019). These compounds have been reported properties by disrupting microbial cell membranes, inhibiting microbial enzymes, and interfering with microbial DNA replication or protein synthesis. The high content of phytochemicals, such as annonaceous acetogenins in *Annona* species, may contribute to their antimicrobial efficacy through various mechanisms, including inhibition of ATP production in microbial cells and disruption of mitochondrial function. The synergistic effects of multiple bioactive compounds in these plants may enhance their antimicrobial activity against various microorganisms. However, further research is needed to fully elucidate the precise mechanisms of antimicrobial activity of *A. muricata, A. squamosa,* and *A. reticulata*.

Table 2. Inhibition zone of dried leaves infusion of three species of *Annona* against *S. aureus* compared to positive control (Chloramphenicol)

Treatments	Inhibition zone (mm)	Percentage
Chloramphenicol (positive control)	22.33	-
Dried leaves infusion of soursop	8.74	39.14
Dried leaves infusion sugar apple	7.1	31.80
Dried leaves infusion of custard apple	10.65	47.69

Table 3. MIC and MLC of dried infusion extracts from Soursop leaves (*A. muricata*), Sugar-Apple (*A. squamosa*), and Custard Apple (*A. reticulata*) against the tested microbes

Extract	MIC (%)	MLC (%)
Soursop leaves (dried infusion)	6.25%	12.5%
Sugar-apple leaves (dried infusion)	6.25%	25%
Custard apple leaves (dried infusion)	6.25%	12.5%

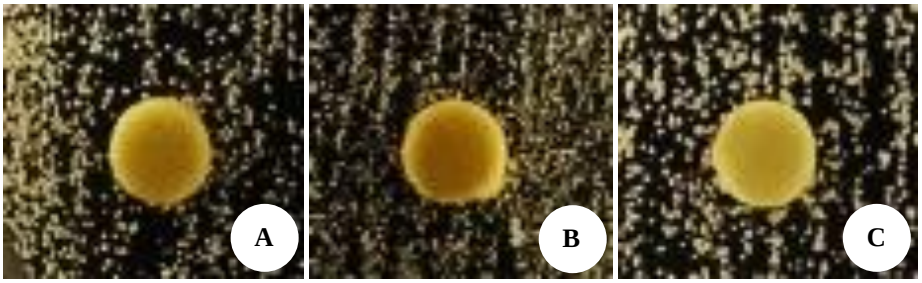


Figure 3. Antimicrobial activity of dried infusion extracts from soursop *leaves (A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*) against *C. albicans*. A. Custard Apple leaves; B. Soursop leaves; C. Sugar-Apple leaves

Table 4. Total polyphenols of dried infusion extracts from soursop leaves (*A. muricata*), sugar apple (*A. squamosa*), and custard apple (*A. reticulata*)

Extract	Polyphenol (mgGAE/mL)
Soursop leaves (Dried infusion)	17.98
Sugar-apple leaves (Dried infusion)	19.79
Custard apple leaves (Dried infusion)	38.97

Table 5. Antioxidant activity of the infusion of dried soursop leaves (*A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*)

Extract	IC_{50} value (µg/mL)
Soursop leaves (Dried infusion)	38.56
Sugar-apple leaves (Dried infusion)	62.49
Custard apple leaves (Dried infusion)	69.28

The correlation of total polyphenols with antimicrobial activity

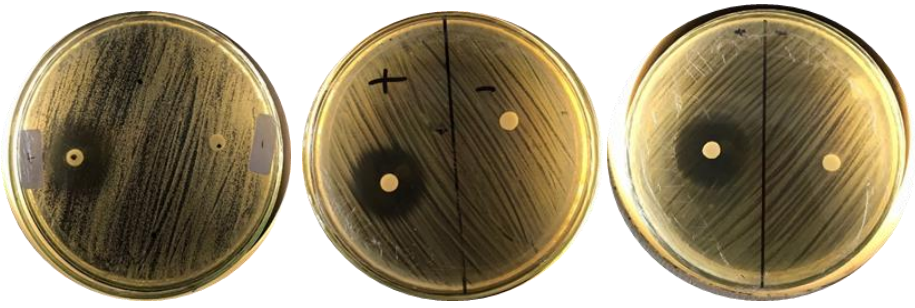
The total phenolic content of soursop leaves *(A. muricata*), sugar apple (*A. squamosa*), and custard apple (*A. reticulata*) were presented in Table 4. Based on Table 4, the results show that the polyphenol content of dried infused soursop leaves is 17.98 mgGAE/mL, followed by infused dried custard apple leaves is 19.79 mgGAE/mL, and infused dried sugar apple leaves is 38.97 mgGAE/mL. The phenolic content of leaf-infused dried sugar apple is higher than that of infused dried soursop and custard apple leaves. It is indicated that infused dried soursop and custard apple leaves contain compounds that have the potential as antioxidants besides phenolic compounds. Terpenoid and alkaloid compounds are compounds with antioxidant activity other than phenolic compounds (Cruz-Casas et al. 2023).

The results showed that polyphenols correlate strongly with antimicrobial activity using diffusion and dilution methods. The results showed that the infused dried custard apple leaf extract has the highest antimicrobial activity. It might be due to the presence of phenols and polyphenols as primary contributors to antibacterial agents. The higher the extract's total phenol content, the higher the antibacterial activity (Dias et al. 2019). Hydroxyl groups of phenolic compounds (OH) influence antibacterial activity in inhibiting bacteria. The level and quantity of hydroxyl functional groups (OH) in the phenol group are related to its level of toxicity to microorganisms. The higher the hydroxylation process, the higher the toxicity.

Total polyphenols and antioxidant activity

The antioxidant activity of the infusion of dried soursop leaves *(A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*) were presented in Table 5. Based on Table 5, the results show that the antioxidant activity of infused dried soursop leaves is classified as very strong (38.56 µg/mL), indicating that infused dried soursop leaves can oxidize DPPH free radicals (Ramalingam and Rajaram 2018). The significant antioxidant activity of the infused dried soursop leaf extract is attributed to its chemical compounds, i.e., flavonoid compounds with an exceptionally high percentage of kaempferol. Hydroxyl groups on kaempferol (3,7,4') were unsaturated bonds C2-C3, carbonyl groups at C-4, and hydroxyl groups at positions 3 and 5 play a crucial role as antioxidants, forming hydrogen bonds with carbonyl groups (Patiño-Ruiz et al. 2020). Furthermore, the antioxidant activity of infused dried sugar apple leaves is classified as strong, with a value of 62.49 µg/mL, in which infused dried sugar apple leaves can oxidize 50% of DPPH free radicals at the concentration of 0.1 mM. Previous research by Werdiningsih and Zahro (2020) stated that the antioxidant activity of sugar apple leaf is classified as strong, with a value of 60.437 µg/mL (Werdiningsih and Zahro 2020). The extraction method also affects the difference in inhibition concentration values (IC_{50}).

The antioxidant activity of infused dried custard apple leaves is classified as strong, with a value of 69.28 µg/mL, indicating that infused dried custard apple leaves can oxidize DPPH free radicals due to phenolic and flavonoid compounds in the infused dried custard apple leaf extract (Ramalingam and Rajaram 2018). According to Werdiningsih and Zahro (2020), the sample's levels of phenolic and flavonoid compounds influence its antioxidant activity. The higher the levels of phenolic and flavonoid compounds, the higher the antioxidant activity (Werdiningsih and Zahro 2020). Some antioxidant compounds also exhibit anti-inflammatory properties.



A **B** **C**

Figure 4. Microbial inhibition zone test area of control treatment: A. *C. albicans*; B. *S. aureus*; C. *E. coli* control

The antioxidant activity of flavonoid compounds is due to the inhibition of the oxidation of arachidonic acid into endoperoxides (Ramalingam and Rajaram 2018). By inhibiting the oxidation of arachidonic acid, it can prevent the formation of reactive oxygen species and chemical mediators that can cause inflammation. Antioxidants can also reduce the activity of lipoxygenase enzymes, preventing the formation of leukotrienes that can inactivate leukocytes, thus triggering inflammation. Terpenoid compounds are known to inhibit inflammation through various mechanisms, one of which is by inhibiting the activity of lipoxygenase and cyclooxygenase enzymes (Hashem et al. 2023).

Carotenoid effects

The carotenoid activity of leaf extract of soursop leaves *(A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*) were presented in Table 6.

Based on Table 6, the results show that soursop *(A. muricata*), sugar apple (*A. squamosa*), and custard apple (*A. reticulata*) leaves contain carotenoids as one of the water-insoluble antioxidants. In the fresh extract of sugar apple leaves, the carotenoid content is 548.84 µmol/g, and the fresh extract of custard apple leaves contains 509.43 µmol/g of carotenoids, while the lowest is in the fresh extract of soursop leaves, which is 431.59 µmol/g. The differences in carotenoid levels are suspected to be due to variations in the compounds and pigments present in each leaf. The carotenoid content in a substance is influenced by various factors such as the environment (food and light intensity), type, and others (Idenyi et al. 2022).

The compound carotenoid is also suspected to influence antioxidant activity. Carotenoids are orange or red pigments consisting of isoprenoid polyene compounds. The color in carotenoids is formed due to a chromophore group characterized by conjugated double bonds in the carotenoid molecule. The more conjugated double bonds, the more intense the carotenoid color, tending towards red or orange (Yun, Yan, and Liu 2022).

Table 6. Carotenoid activity of leaf extract from soursop leaves (*A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*)

Extract	Carotenoid activity (µmol/g)
Soursop leaves (fresh extract)	431.59
Sugar-apple leaves (fresh extract)	548.84
Custard apple leaves (fresh extract)	509.43

In conclusion, the three extracts of soursop leaves (*A. muricata*), sugar-apple (*A. squamosa*), and custard apple (*A. reticulata*) had significantly different antibacterial activity against *S. aureus*, and the highest was the infused dried soursop leaf extract (10.65 mm). There were no inhibition zones against *E. coli*. The Minimum Inhibitory Concentration (MIC) of the leaf infusions of soursop and custard apple leaf extracts against S. aureus was 6.25%, and the Minimum Lethal Concentration (MLC) was 12.5%. The MIC of sugar apple leaf extract against *S.aureus* was 6.25% with an MLC of 25%. The inhibition potency of the infused dried custard apple leaf extract is 48.58% against *S. aureus* compared to the positive control chloramphenicol (0.1 mg/mL), 24.57% against *E. coli*, and 28.70% against *C. albicans* compared to the positive control fluconazole (0.1 mg/mL). The highest antioxidant activity was in the infused dried soursop leaf extract with an IC_{50} value of 38.56 µg/mL. The infused dried custard apple leaf extract had the highest polyphenol content (38.97 mgGAE/mL). The highest carotenoid content was in the fresh extract of sugar apple leaves (548.84 µmol/g). These infusions may be used for multidrug-resistant pathogens. Additionally, The antioxidant properties of *Annona* extracts offer further benefits by mitigating oxidative stress. Due to their excellent potency, these leaf infusions may be developed as an effective and sustainable source of antimicrobial and antioxidant agents against AMR to address urgent global health challenges.

ACKNOWLEDGEMENTS

The author gives a big thanks to the lectures of the advisor, who provided advice, suggestions, and guidance so that the author could complete the research and prepare this article. This research occurred without funding from any institution.

REFERENCES

Ahmad AS, Sae-Ieaw T, Ma L, Zhang B, Benjakul S. 2024. Combined effect of prior pulsed electric field, dechlorophyllized soursop leaf extract and vacuum impregnation on melanosis inhibition and shelf-life extension of refrigerated Pacific white shrimp. Food Control 163: 110494. DOI: 10.1016/j.foodcont.2024.110494.

Ahmad Shiekh K, Olatunde OO, Zhang B, Huda N, Benjakul S. 2021. Pulsed electric field assisted process for extraction of bioactive

compounds from custard apple (*Annona squamosa*) leaves. Food Chem 359: 129976. DOI: 10.1016/j.foodchem.2021.129976.
Ahmed B, Floc'h J-B, Lahrach Z, Hijri M. 2021. Phytate and microbial suspension amendments increased soybean growth and shifted microbial community structure. Microorganisms 9 (9): 1803. DOI: 10.3390/microorganisms9091803.
Balouiri M, Sadiki M, Ibnsouda SK. 2016. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 6 (2): 71-79. DOI: 10.1016/j.jpha.2015.11.005.
Bitwell C, Indra SS, Luke C, Kakoma MK. 2023. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Sci Afr 19: e01585. DOI: 10.1016/j.sciaf.2023.e01585.
Chowdhury SS, Tareq AM, Tareq SM, Farhad S, Sayeed MA. 2021. Screening of antidiabetic and antioxidant potential along with phytochemicals of *Annona* genus: A review. Futur J Pharm Sci 7: 144. DOI: 10.1186/s43094-021-00300-9.
Cruz-Casas DE, Aguilar CN, Ascacio-Valdés JA, Rodríguez-Herrera R, Chávez-González ML, Flores-Gallegos A.C. 2023. Bioactive protein hydrolysates obtained from amaranth by fermentation with lactic acid bacteria and *Bacillus* species. Heliyon 9 (2): e13491. DOI: 10.1016/j.heliyon.2023.e13491.
Dias WLF, do Vale Junior EP, das Dores Alves de Oliveira M, Barbosa YLP, do Nascimento Silva J, da Costa Júnior JS, de Almeida PM, Martins FA. 2019. Cytogenotoxic effect, phytochemical screening, and antioxidant potential of *Jatropha mollissima* (Pohl) Baill leaves. S Afr J Bot 123: 30-35. DOI: 10.1016/j.sajb.2019.02.007.
Dos Santos ÉL, Santana AC, Micheletti AC, Freire TV, Guterres ZR, Yoshida NC. 2023. Metabolomic profiling and assessment of antimicrobial, antioxidant, and genotoxic potential of *Unonopsis guatterioides* R.E.Fr. (Annonaceae) fruits. Arab J Chem 16 (10): 105133. DOI: 10.1016/j.arabjc.2023.105133.
El-Belely EF, Farag MMS, Said HA, Amin AS, Azab E, Gobouri AA, Fouda A. 2021. Green synthesis of zinc oxide nanoparticles (Zno-nps) using *Arthrospira platensis* (class: Cyanophyceae) and evaluation of their biomedical activities. Nanomaterials 11 (1): 95. DOI: 10.3390/nano11010095.
Fonmboh DJ, Abah ER, Fokunang TE, Herve B, Teke GN, Rose NM, Borgia NN, Fokunang LB, Andrew BN, Kaba N, Bathelemy N, Ntungwen FC. 2020. An overview of methods of extraction, isolation and characterization of natural medicinal plant products in improved traditional medicine research. Asian J Res Med Pharm Sci 9 (2): 31-57. DOI: 10.9734/ajrimps/2020/v9i230152.
Haruna MF, Kenta AM, Herawati. 2022. Medicinal plants used by the community of Lipulalongo Village, Banggai Laut District, Central Sulawesi, Indonesia. Asian J Ethnobiol 5: 62-68. DOI: 10.13057/asianjethnobiol/y050107.
Hashem AH, Attia MS, Kandil EK, Fawzi MM, Abdelrahman AS, Khader MS, Khodaira MA, Emam AE, Goma MA, Abdelaziz AM. 2023. Bioactive compounds and biomedical applications of endophytic fungi: A recent review. Microb Cell Fact 22: 107. DOI: 10.1186/s12934-023-02118-x.
Herrera-Calderon O, Enciso-Roca E, Pari-Olarte B, Arroyo-Acevedo J. 2016. Phytochemical screening, antioxidant activity and analgesic effect of *Waltheria ovata* Cav. roots in mice. Asian Pac J Trop Dis 6 (12): 1000-1003. DOI: 10.1016/S2222-1808(16)61172-6.
Idenyi JN, Eya JC, Nwankwegu AS, Nwoba EG. 2022. Aquaculture sustainability through alternative dietary ingredients: Microalgal value-added products. Eng Microbiol 2 (4): 100049. DOI: 10.1016/j.engmic.2022.100049.
Jemikalajah J, Enwa FO, Chiedozie EC. 2021. Antibacterial activity of *Annona muricata* (soursop) leaf extract) on *Escherichia coli* and *Pseudomonas aeruginosa*. Turk J Physiother Rehabil 32 (3): 13918-13923.
Kahraman C, Topcu G, Bedir E, Tatli II, Ekizoglu M, Akdemir ZS. 2019. Phytochemical screening and evaluation of the antimicrobial and antioxidant activities of *Ferula caspica* M. Bieb. extracts. Saudi Pharm J 27 (4): 525-531. DOI: 10.1016/j.jsps.2019.01.016.
Kavithaa K, Paulpandi M, Ponraj T, Murugan K, Sumathi S. 2016. Induction of intrinsic apoptotic pathway in human breast cancer (MCF-7) cells through facile biosynthesized zinc oxide nanorods. Karbala Intl J Mod Sci 2: 46-55. DOI: 10.1016/j.kijoms.2016.01.002.
Kumar M, Changan S, Tomar M, Prajapati U, Saurabh V, Hasan M, Sasi M, Maheshwari C, Singh S, Dhumal S, Radha, Thakur M, Punia S, Satankar V, Amarowicz R, Mekhemar M. 2021. Custard apple (*Annona squamosa L.*) leaves: Nutritional composition, phytochemical profile, and health-promoting biological activities. Biomolecules 11 (5): 614. DOI: 10.3390/biom11050614.
Kumari A, Sharan L, Patnaik A. 2021. *Annona reticulata L.* phytoconstituents and pharmacology: A review. Intl J Ecol Environ Sci 3 (3): 159-165.
Kuppusamy S, Venkateswarlu K, Megharaj M. 2020. Examining the polyphenol content, antioxidant activity and fatty acid composition of twenty-one different wastes of fruits, vegetables, oilseeds and beverages. SN Appl Sci 2: 673. DOI: 10.1007/s42452-020-2441-9.
Lingaraju K, Naika HR, Nagabhushana H, Nagaraju G. 2019. *Euphorbia heterophylla* (L.) mediated fabrication of ZnO NPs: Characterization and evaluation of antibacterial and anticancer properties. Biocatal Agric Biotechnol 18: 100894. DOI: 10.1016/j.bcab.2018.10.011.
Mariychuk R, Porubská J, Ostafin M, Čaplovičová M, Eliašová A. 2020. Green synthesis of stable nanocolloids of monodisperse silver and gold nanoparticles using natural polyphenols from fruits of *Sambucus nigra* L. Appl Nanosci 10: 4545-4558. DOI: 10.1007/s13204-020-01324-y.
Medda S, Hajra A, Dey U, Bose P, Mondal NK. 2015. Biosynthesis of silver nanoparticles from *Aloe vera* leaf extract and antifungal activity against *Rhizopus* sp. and *Aspergillus* sp. Appl Nanosci 5: 875-880. DOI: 10.1007/s13204-014-0387-1.
Molyneux P. 2004. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 50: 211-219.
Ngbolua K-T-N, Inkoto CL, Bongo GN, Moke LE, Lufuluabo LG, Ashande CM, Tshibangu DST, Tshilanda DD, Mpiana PT. 2018. Phytochemistry and bioactivity of *Annona reticulata* L. (Annonaceae): A mini-review. S Asian Res J Nat Prod 1 (1): 1-11.
Nurmiati N, Periadnadi P, Alamsyah F, Sapalina F. 2018. Characterization and potential of acid fermentative and proteolytic natural microflora in several products of traditional Dadih from Lembah Gumanti District West Sumatra, Indonesia. Intl J Curr Microbiol Appl Sci 7 (3): 3151-3163. DOI: 10.20546/ijcmas.2018.703.364.
Patiño-Ruiz D, Sánchez-Botero L, Tejeda-Benitez L, Hinestroza J, Herrera A. 2020. Green synthesis of iron oxide nanoparticles using *Cymbopogon citratus* extract and sodium carbonate salt: Nanotoxicological considerations for potential environmental applications. Environ Nanotechnol Monit Manag 14: 100377. DOI: 10.1016/j.enmm.2020.100377.
Rajendran R, Mani A. 2020. Photocatalytic, antibacterial and anticancer activity of silver-doped zinc oxide nanoparticles. J Saudi Chem Soc 24 (12): 1010-1024. DOI: 10.1016/j.jscs.2020.10.008.
Ramalingam V, Rajaram R. 2018. Enhanced antimicrobial, antioxidant and anticancer activity of *Rhizophora apiculata*: An experimental report. 3 Biotech 8 (4): 200. DOI: 10.1007/s13205-018-1222-2.
Ramyabharathi SA, Meena KS, Rajendran L, Raguchander T, Jonathan EI. 2020. Potential of a rhizobacterium *Bacillus subtilis* (Bbv 57) on *Fusarium oxysporum* f. sp. *gerberae* and *Meloidogyne incognita* infecting Gerbera grown in protected cultivation. Eur J Plant Pathol 158 (3): 615-632. DOI: 10.1007/s10658-020-02087-6.
Rohmah J, Margareta FA. 2023. Antioxidant activity, total flavonoids and total phenolics content of infusion, and decoction of White Agathi (*Sesbania grandiflora* (L.) Pers.) leaves extracts. AIP Conf Proc 2642: 070001. DOI: 10.1063/5.0110918.
Shrestha R, Thenissery A, Khupse R, Rajashekara G. 2023. Strategies for the preparation of Chitosan derivatives for antimicrobial, drug delivery, and agricultural applications: A review. Molecules 28 (22): 7659. DOI: 10.3390/molecules28227659.
Tiwari V, Mishra N, Gadani K, Solanki PS, Shah NA, Tiwari M. 2018. Mechanism of anti-bacterial activity of zinc oxide nanoparticle against Carbapenem-Resistant *Acinetobacter baumannii*. Front Microbiol 9: 1218. DOI: 10.3389/fmicb.2018.01218.
Uchegbu RI, Ukpai KU, Iwu IC, Akalazu JN. 2017. Evaluation of the antimicrobial activity and chemical composition of the leaf extract of *Annona muricata* Linn (Soursop) grown in Eastern Nigeria. Arch Curr Res Intl 7 (1): 1-7. DOI: 10.9734/acri/2017/31929.
Viñas-Ospino A, Panić M, Radojčić- Redovniković I, Blesa J, Esteve MJ. 2023. Using novel hydrophobic deep eutectic solvents to improve a sustainable carotenoid extraction from orange peels. Food Biosci 53: 102570. DOI: 10.1016/j.fbio.2023.102570.
Vishvakarma P, Mandal S, Verma A, Author-Prabhakar VCA. 2023. A review on current aspects of nutraceuticals and dietary supplements. Intl J Pharm Prof Res 14: 78-91. DOI: 10.48165/ijppronline.2023.14107.
Wang Y, Zhang M, Zhan C. 2023. A review on the nested distribution pattern (nestedness): Analysis methods, mechanisms and conservation

implications. Biodivers Sci 31: 1-17. DOI: 10.17520/biods.2023314. [Chinese]

Weng Z, Zeng F, Wang M, Guo S, Tang Z, Itagaki K, Lin Y, Shen X, Cao Y, Duan J-A, Wang F. 2024. Antimicrobial activities of lavandulylated flavonoids in *Sophora flavences* against methicillin-resistant *Staphylococcus aureus* via membrane disruption. J Adv Res 57: 197-212. DOI: 10.1016/j.jare.2023.04.017.

Werdiningsih W, Zahro A. 2020. Determination of flavonoid and phenol levels from Srikaya (*Annona squamosa* L) leaves and their activity as antioxidants. Jurnal Wiyata 7 (2): 157-170. [Indonesian]

Yun D, Yan Y, Liu J. 2022. Isolation, structure and biological activity of polysaccharides from the fruits of *Lycium ruthenicum* Murr: A review. Carbohydr Polym 291: 119618. DOI: 10.1016/j.carbpol.2022.119618.